SHORT COMMUNICATION

Phytochemical cues affect hunting-site choices of a nursery web spider (*Pisaura mirabilis*) but not a crab spider (*Misumena vatia*)

Robert R. Junker[1,3], Simon Bretscher[1], Stefan Dötterl[2] and Nico Blüthgen[1]: [1]Department of Animal Ecology and Tropical Biology, Biozentrum, University of Würzburg, Germany. E-mail: bluethgen@biozentrum.uni-wuerzburg.de; [2]Department of Plant Systematics, University of Bayreuth, Germany

Abstract. Predaceous arthropods such as spiders are often adapted to hunting sites where their hunting success is greatest. We investigated the responses of two spiders to phytochemical cues that they potentially experience while hunting on leaves or flowers, and how these cues could influence their decisions where to forage. We compared the behavior of two sit-and-wait predators, *Pisaura mirabilis* and *Misumena vatia*, which hunt predominantly in the vegetation or on flowers, respectively. In choice tests, *P. mirabilis* frequently preferred leaves and leaf extracts to flowers and floral extracts and avoided substrates treated with the common floral scents β-caryophyllene and nerolidol (sesquiterpenes) in natural concentrations. In contrast, *M. vatia* did not show any preferences for any of the substrates and treatments offered. The lack of responses by *M. vatia* contrasts with earlier studies on another crab spider species (*Thomisus spectabilis*) that used phytochemical cues as a guide to rewarding flowers. The avoidance of many flowers, their extracts, and the floral scent compounds by *P. mirabilis* suggests that these cues may prevent the visitation by this and other generalised predators that potentially decrease the pollination success of a plant.

Keywords: Deterrence, optimal foraging, secondary metabolites

An underlying assumption of many optimal foraging models is that animals are behaviorally, morphologically and physiologically adapted to maximize their net rate of energy intake (Schoener 1971; Cowie 1977). A behavioral adaptation of predaceous animals is to choose foraging patches that are frequently visited by prey or to which the animals are best adapted (Krebs et al. 1974; Shafir & Roughgarden 1998). Some crab spiders, for example, show adaptations as sit-and-wait predators on flowers: they are able to change color for camouflage and enhance the attractiveness of flowers for pollinators due to their ultraviolet contrast against petals (Heiling et al. 2003). The high specialization on flowers by crab spiders is also reflected in a relatively narrow prey spectrum, which is limited to common flower visitor taxa (Nentwig 1986). Other non-web-building spiders hunt or ambush predominantly in the vegetation and thus capture a broader spectrum of prey taxa (Nentwig 1986). To benefit from their adaptations to different plant structures (vegetative versus reproductive) or to specific visitors of these structures, spiders need to perceive and thus recognize those structures. Heiling et al. (2004) have shown that crab spiders (*Thomisus spectabilis*) use visual and olfactory flower cues for patch choice.

We experimentally tested for substrate choice behavior and a role of phytochemicals in two non-web-building spiders that utilize different plant parts as hunting sites: the crab spider *Misumena vatia* (Thomisidae), which typically sits and waits on flowers to catch flower visitors, and the nursery web spider *Pisaura mirabilis* (Pisauridae), which hunts in the vegetation. In concordance with their lifestyle, we expected *M. vatia* to be attracted to flower cues, while *P. mirabilis* may prefer leaves.

Between June and August 2008, we caught *M. vatia* and *P. mirabilis* spiders on fallow lands in Würzburg, Germany. We collected fifty-eight individuals of *M. vatia* on flowers of *Achillea millefolium*, *Aegopodium podagraria*, *Leucanthemum vulgare*, *Saponaria officinalis*, *Solidago canadensis*, *Trifolium pratense*, *Tripleurospermum maritimum*, while we collected all but one of 41 *P. mirabilis* from the

vegetation (one individual was collected from an *Achillea millefolium* flower). We kept the spiders individually in small plastic containers in a climate chamber under long day conditions (day:night = 14:10 h, 26:19° C) and fed them with flies twice a week and continuously provided water as a small drop. We picked the plants used for the laboratory experiments in the same area.

In pair-wise choice tests, spiders were able to choose between different substrates including flowers vs. leaves of the same plant species (Experiment I), filter papers with extracts of flowers vs. extracts of leaves of the same plant species (Experiment II) and filter papers treated with synthetic floral scent compounds vs. unscented controls (Experiment III). The principal setup of these experiments (I–III) was the same: we placed individual spiders on pieces of cork representing "islands" (ca 30 cm²) in water-filled bowls, preventing spiders from escaping. On each of these islands, we attached two wooden sticks (height = 140 mm, diam. = 3 mm) in an upright position and attached the different substrates used in the tests to the tip of these sticks. The distance between the substrates (ca 1 cm) was chosen to be close enough that the spiders could freely change between the substrates without descending to the islands but large enough that spiders were forced to make a choice. Neon lamps from above illuminated the whole setup. After spiders were placed on the islands, we observed them for 1 h, recording their position on either substrate every 3 min. We used individual spiders for several tests but not repeatedly for the same treatment.

Experiment I: We placed freshly picked flowers and leaves from *Achillea millefolium*, *Centaurea cyanus*, *Tanacetum vulgare* (all Asteraceae), *Medicago sativa* (Fabaceae) and *Saponaria officinalis* (Caryophyllaceae) in small water-filled vases. The vases were 1.5 ml standard microcaps, and we attached them on top of the wooden sticks. In each pair-wise test (flower vs. leaf of the same plant species), we adjusted the number of leaves and flowers or inflorescences so that both substrates represented approximately the same area, providing sufficient space for spiders to sit on.

Experiment II: We used the same five plant species to prepare leaf and flower extracts. We placed freshly chopped plant material into an extraction thimble and continuously extracted it with 50 ml *n*-hexane in a Soxhlet apparatus for three hours at a temperature of 85° C

[3]Current address: Heinrich-Heine-University of Düsseldorf, Department Biology, Institute of Sensory Ecology, Universitätsstraβe 1, 40225 Düsseldorf, Germany

Table 1.—Generalized linear models (GLM with quasibinomial error distribution) of the proportional choices for flowers, flower extracts or synthetic compounds in *Pisaura mirabilis* and *Misumena vatia*: a) trials using fresh plant material (flowers versus leaves, Experiment I) and extracts of flowers *versus* extracts of leaves (Experiment II). Factor "treatment" refers to trials using fresh plant materials or extracts thereof. b) Trials using synthetic scent compounds versus the acetone-only treatment (Experiment III). Starting with the full model containing all explanatory parameters, each reduced model was compared with the previous one with a X^2 test resulting in deviance, number of degrees of freedom (df_1), residual degrees of freedom (df_2) and significance (P) for each parameter.

Parameter	Deviance	df_1	df_2	P
a)				
Spider species * plant species * treatment	4.53	9	288	0.58
Treatment	0.00	1	297	0.99
Spider species * plant species	5.48	4	298	0.053
Plant species	7.95	4	302	< 0.01
Spider species	14.25	1	306	< 0.001
Residual error	199.85			
Total	232.06			
b)				
Spider species * substance * concentration	2.54	3	226	0.27
Concentration	0.08	1	227	0.73
Spider species * substance	5.41	5	232	0.14
Substance	8.94	5	237	0.014
Spider species	4.37	1	238	< 0.01
Residual error	163.61			
Total	184.94			

(Baysal & Starmans 1999). We removed the solvent under vacuum and resolved the extract in acetone. We determined the volume of acetone as $0.75 \cdot$ g dry weight $\cdot$ 200µl acetone and applied aliquots of the extract (200 µl) on round filter papers (diameter = 35 mm) that were attached on top of the wooden sticks. Thus, the extract was applied to filter papers with a mass of 75% of the plant dry weight to account for losses of the extract during the process. We tested flower and leaf extracts of each plant species again pair-wise.

In order to determine those compounds in the extracts that frequently occur in flower and leaf scents (Knudsen et al. 2006), we analysed the extracts using a Varian 3800 gas chromatograph (GC) fitted with a 1079 injector and a ZB-5 column (5% phenyl polysiloxane; length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 µm; Phenomenex) and a Varian Saturn 2000 mass spectrometer. We placed 1 µl of the samples into a quartz vial in the injector port of the GC by means of the ChromatoProbe kit (Amirav & Dagan 1997). The injector split vent was opened, and the injector was heated at 40° C to flush any air from the system. After 2 min, the split vent was closed and the injector heated at 200° C min⁻¹, then held at 260° C until the end of the run. The split vent was again opened after 4.5 min. Electronic flow control was used to maintain a constant helium carrier gas flow rate (1.0 ml min⁻¹). The GC oven temperature was held for 4.5 min at 40° C, then increased by 6° C min⁻¹ to 300° C, and held for 15 min at this temperature. Mass spectra were taken at 70 eV with a scanning speed of one scan per second from m/z 30 to 650. We analyzed the data as described elsewhere (Dötterl et al. 2009), and used an internal standard (3-chloro-4-methoxytoluene) for quantification.

Experiment III: Since we expected that the phytochemical cues to which spiders respond are not specific to certain plant species, we used commonly occurring flower and leaf scent compounds that were also present in the extracts for subsequent bioassays. Among the compounds identified in the samples, we selected benzaldehyde (benzenoid), 1-hexanol, *cis*-3-hexen-1-ol, *cis*-3-hexen-1-yl acetate (all aliphatics), limonene, linalool (monoterpenoids), β-caryophyllene and nerolidol (mixture of *cis*- and *trans*-isomers, sesquiterpenoids), because these compounds are common and widespread floral scent compounds (Knudsen et al. 2006). 1-hexanol, *cis*-3-hexen-1-ol and *cis*-3-hexen-1-yl acetate are also common green leaf volatiles (Pare & Tumlinson 1999); *cis*-3-hexen-1-ol and *cis*-3-hexen-1-yl acetate were

tested with *P. mirabilis* only. We dissolved substances in acetone and applied them in different amounts starting with 0.01 mMol per filter paper. In cases where a substance affected the choice of one of the spider species in this initial concentration, we subsequently reduced the amount (0.005, 0.0025, and 0.00125 mMol per filter paper) in order to explore concentration-dependent effects. We attached the scented filter papers (treatment) and filter papers treated only with acetone (controls) on top of the wooden sticks. After approximately 10 min, after the solvent had evaporated, a trial started.

Each trial (1-h period) yielded up to 20 observations from which the proportion of observations on flowers (Experiment I), flower extracts (II) or scented filter papers (III) was obtained, disregarding observations during which the spider was not present on one of the substrates. Some spiders spent time on the islands, while others did not leave it during the entire period (*P. mirabilis*: 3.0% of all trials, *M. vatia*: 7.3%); these rare events were not included in the calculation of the proportion. We performed generalized linear models (GLM) with quasibinomial error distribution (accounting for the overdispersed data) in order to explore the parameters influencing the spiders' choice. We analysed the tests with fresh plant material (Experiment I) and extracts (Experiment II) in one GLM, with the proportion of observations on flowers or flower extracts as response variable and spider species, plant species and treatment (i.e., fresh plant material or extracts) as explanatory variables. In the GLM for tests with floral scent compounds (Experiment III), we used spider species, substance and concentration (mMol) as explanatory variables. Beginning with the full model, we reduced the models stepwise and compared them to the previous one with a X^2 test (Crawley 2005). Prior to the stepwise statistical analysis, we compared the full model to a null model (model with no explanatory variables) to validate the overall effect of the combined parameters. We tested individual parameters only if the full model had significantly more explanatory power than the null model (see Mundry & Nunn 2009). Additionally, we individually tested the proportions against the null hypothesis (assuming equal visitation of both treatment and control; i.e., proportion = 0.5) with a Wilcoxon test. All statistical analyses were performed using R 2.4.0 (R Development Core Team 2009).

In 93.3 and 97.5% of all trials with *M. vatia* and *P. mirabilis*, respectively, the spider chose one of the substrates within the first
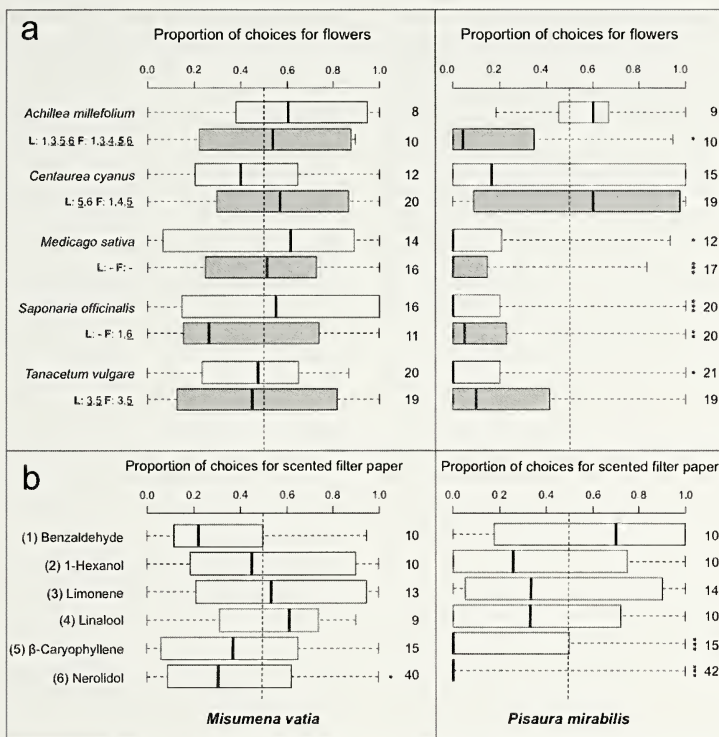
Figure 1.—Dual choices of *Pisaura mirabilis* and *Misumena vatia* between flowers and leaves, extracts or synthetic compounds. Choices were measured as proportion of choices for flowers and their extracts (a. experiments I and II) or scents (b. Experiment III) of the total time on both treatments. Significant deviation from an equal proportion of visits on flowers and leaves, or scent and control (i.e., proportion = 0.5) is indicated by asterisks using paired Wilcoxon rank sum test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Sample sizes are given next to each box plot. a) White boxes show trials with fresh plant material (flowers vs. leaves), gray boxes flower vs. leaf extracts. Leaf (L) and flower (F) extracts often contained one or more substances used in the bioassay, which are listed below each species name. Numbers correspond to the substance code below (see b). Concentrations of substances in plant materials are labelled as follows: plain numbers: $1 \cdot 10^{-5}$–0.01 mMol g^{-1} dry weight; underlined numbers: 0.011–10 mMol g^{-1}; underlined, boldfaced and italic numbers: > 10 mMol g^{-1}. (b) Results of trials using synthetic floral scent compounds tested against the acetone-only control.

8 min. Once a spider climbed up a wooden stick, it rarely descended to islands again. While *M. vatia* often changed the substrates during the trial (3.0 ± 0.2 times, mean ± SE), *P. mirabilis* was less likely to switch, with only 0.8 ± 0.2 changes of the substrate per trial. The responses to fresh plant material (Experiment I) were usually consistent with responses to extracts of the same plant species (Experiment II) for both species of spider, but the spiders' choices between leaves and flowers differed strongly between plants (Table 1a). *P. mirabilis* strongly preferred leaves over flowers (and their extracts) in three out of five plant species, whereas *M. vatia* did not show any preferences (Table 1a and Fig. 1a).

In trials where spiders were allowed to choose between filter paper treated with scent compounds and acetone-treated filter paper (Experiment III), the choices depended on the particular substance and spider species. Overall, the concentration of the compounds did not affect the spiders' choices (Table 1b). Similar to the previous tests,

M. vatia was less selective than *P. mirabilis* (Table 1b and Fig. 1b). *M. vatia* avoided filter paper treated with nerolidol, and *P. mirabilis* avoided both nerolidol and β-caryophyllene (Fig. 1b). *P. mirabils* behavior was not affected by the green leaf volatiles *cis*-3-hexen-1-ol and *cis*-3-hexen-1-yl acetate ($V \leq 50.5$, $P \geq 0.37$, Wilcoxon test). Large amounts of nerolidol occurred in floral extracts of *S. officinalis*, and β-caryophyllene in *A. millefolium*. These substances may have triggered the preference of *P. mirabilis* for leaves and leaf extracts in *S. officinalis*, and for leaf extracts of *A. millefolium* over the respective flowers or floral extracts (Fig. 1). Living flowers of *A. millefolium* were not avoided by *P. mirabilis*, suggesting that some substances were dissolved from the plant tissue and were thus present in the extracts that were not emitted by fresh plant material or were emitted in a lesser amount.

The results of our study imply that *P. mirabilis* perceive phytochemical cues and use them to decide where to ambush for

prey. In *M. vatia*, behavioral responses to these cues were much less pronounced, and the crab spiders only responded weakly to the sesquiterpene nerolidol. We had expected that *M. vatia* would prefer flowers and their extracts over leaves and their extracts, since other crab spiders (*Thomisus spectabilis*) positively responded to floral odors (Heiling et al. 2004). Crab spiders including *M. vatia* were shown to prefer fully open and functional flowers (anthesis) over senescent ones (Chien & Morse 1998; Heiling & Herberstein 2004a) and therefore have the same preferences as pollinators and use olfactory in addition to visual cues (Heiling et al. 2004). However, we could not confirm positive responses to floral odors or compounds thereof. Greco and Kevan (1994; 2001) also reported no discrimination between leaves and flowers by the same spider species. It was shown that *M. vatia* remains longer on flowers that are frequented by pollinators (Chien & Morse 1998; Morse 2000a) and on flowers that they have experienced before (Morse 2000b). We used picked flowers (i.e., not the preferred state of the flowers) that were not visited by insects, which may contribute to a lack of preferences.

The preference for leaves over flowers in *P. mirabilis* may either result from an attraction to leaves or from an avoidance of flower secondary metabolites. The trials with individual substances are consistent with the latter and suggest that floral scents or perhaps other non-volatile metabolites have a deterrent effect on this spider. Plant volatiles emitted by flowers and leaves were shown to repel or deter various arthropods (Pichersky & Gershenzon 2002; Gershenzon & Dudareva 2007; Junker & Blüthgen 2008; Kant et al. 2009; Unsicker et al. 2009; Willmer et al. 2009; Junker & Blüthgen 2010). Therefore, it is likely that the floral repellence of this spider represents a typical response of a broad spectrum of generalised predators and other taxa that are not specifically adapted to flowers.

Crab spiders are predators that exploit the mutualism between flowers and pollinators and thereby have detrimental effects on pollination and consequently reproduction of plants (Dukas 2001; Dukas & Morse 2003; Heiling & Herberstein 2004b; Reader et al. 2006; Goncalves-Souza et al. 2008; Ings & Chittka 2008; Brechbühl et al. 2010). Chemical floral cues that prevented predators such as spiders and other floral antagonists from visiting flowers and simultaneously attracted pollinators would maximize the plants' reproductive success (Brown 2002; Irwin et al. 2004; Junker & Blüthgen 2008). Animals that depend on floral resources (obligate flower visitors) are able to tolerate defensive floral scent compounds and even use them as host-finding cues, while facultative flower visitors are not able to (Junker & Blüthgen 2010). The results of the present study suggest such a dichotomy, in which an obligate flower visitor (*M. vatia*) is adapted to flowers as a place to sit and wait for prey, which may include a tolerance against otherwise defensive floral compounds. In contrast, *P. mirabilis* is adapted to use the vegetative plant parts as hunting sites and may not have been subjected to a selective pressure to tolerate the same compounds.

ACKNOWLEDGMENTS

LITERATURE CITED

Amirav, A. & S. Dagan. 1997. A direct sample introduction device for mass spectrometry studies and gas chromatography mass spectrometry analyses. European Mass Spectrometry 3:105–111.

Baysal, T. & D.A.J. Starmans. 1999. Supercritical carbon dioxide extraction of carvone and limonene from caraway seed. Journal of Supercritical Fluids 14:225–234.

Brechbühl, R., C. Kropf & S. Bacher. 2010. Impact of flower-dwelling crab spiders on plant-pollinator mutualisms. Basic and Applied Ecology 11:76–82.

Brown, K. 2002. A compromise on floral traits. Science 298:45–46.

Chien, S.A. & D.H. Morse. 1998. The roles of prey and flower quality in the choice of hunting sites by adult male crab spiders *Misumena vatia* (Araneae, Thomisidae). Journal of Arachnology 26:238–243.

Cowie, R.J. 1977. Optimal foraging in great tits (*Parus major*). Nature 268:137–139.

Crawley, M.J. 2005. Statistics - An Introduction Using R. Wiley, Chichester, UK.

Dötterl, S., A. Jürgens, L. Wolfe & A. Biere. 2009. Disease status and population origin effects on floral scent: potential consequences for oviposition and fruit predation in a complex interaction between a plant, fungus, and noctuid moth. Journal of Chemical Ecology 35:307–319.

Dukas, R. 2001. Effects of perceived danger on flower choice by bees. Ecology Letters 4:327–333.

Dukas, R. & D.H. Morse. 2003. Crab spiders affect flower visitation by bees. Oikos 101:157–163.

Gershenzon, J. & N. Dudareva. 2007. The function of terpene natural products in the natural world. Nature Chemical Biology 3:408–414.

Goncalves-Souza, T., P.M. Omena, J.C. Souza & G.Q. Romero. 2008. Trait-mediated effects on flowers: artificial spiders deceive pollinators and decrease plant fitness. Ecology 89:2407–2413.

Greco, F. & P.G. Kevan. 1994. Contrasting patch choosing by anthophilous ambush predators: vegetation and floral cues for decisions by a crab spider (*Misumena vatia*) and males and females of an ambush bug (*Phymata americana*). Canadian Journal of Zoology 72:1583–1588.

Heiling, A.M., K. Cheng & M.C. Herberstein. 2004. Exploitation of floral signals by crab spiders (*Thomisus spectabilis*, Thomisidae). Behavioral Ecology 15:321–326.

Heiling, A.M. & M.E. Herberstein. 2004a. Floral quality signals lure pollinators and their predators. Annales Zoologici Fennici 41:421–428.

Heiling, A.M. & M.E. Herberstein. 2004b. Predator–prey coevolution: Australian native bees avoid their spider predators. Proceedings of the Royal Society London 271:196–198.

Heiling, A.M., M.E. Herberstein & L. Chittka. 2003. Pollinator attraction - Crab-spiders manipulate flower signals. Nature 421: 334–334.

Ings, T.C. & L. Chittka. 2008. Speed-accuracy tradeoffs and false alarms in bee responses to cryptic predators. Current Biology 18:1520–1524.

Irwin, R.E., L.S. Adler & A.K. Brody. 2004. The dual role of floral traits: Pollinator attraction and plant defense. Ecology 85:1503–1511.

Junker, R.R. & N. Blüthgen. 2008. Floral scents repel potentially nectar-thieving ants. Evolutionary Ecology Research 10:295–308.

Junker, R.R. & N. Blüthgen. 2010. Floral scents repel facultative flower visitors, but attract obligate ones. Annals of Botany 105: 777–782.

Kant, M.R., P.M. Bleeker, M.V. Wijk, R.C. Schuurink & M.A. Haring. 2009. Plant volatiles in defence. Pp. 613–666. *In* Advances in Botanical Research, Volume 51. (L.C. van Loon, ed.). Academic Press, Burlington, Massachusetts.

Kevan, P. & F. Greco. 2001. Contrasting patch choice behaviour by immature ambush predators, a spider (*Misumena vatia*) and an insect (*Phymata americana*). Ecological Entomology 26:148–153.

Knudsen, J.T., R. Eriksson, J. Gershenzon & B. Stahl. 2006. Diversity and distribution of floral scent. Botanical Review 72:1–120.

Krebs, J.R., J.C. Ryan & E.L. Charnov. 1974. Hunting by expectation or optimal foraging? A study of patch use by chickadees. Animal Behaviour 22:953–964.

Morse, D.H. 2000a. Flower choice by naive young crab spiders and the effect of subsequent experience. Animal Behaviour 59:943–951.

Morse, D.H. 2000b. The role of experience in determining patch-use by adult crab spiders. Behaviour 137:265–278.

Mundry, R. & C.L. Nunn. 2009. Stepwise model fitting and statistical inference: turning noise into signal pollution. American Naturalist 173:119–123.

Nentwig, W. 1986. Non-webbuilding spiders - prey specialists or generalists. Oecologia 69:571–576.

Pare, P.W. & J.H. Tumlinson. 1999. Plant volatiles as a defense against insect herbivores. Plant Physiology 121:325–331.

Pichersky, E. & J. Gershenzon. 2002. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Current Opinion in Plant Biology 5:237–243.

Reader, T., A.D. Higginson, C.J. Barnard, F.S. Gilbert & B.E.F. Course. 2006. The effects of predation risk from crab spiders on bee foraging behavior. Behavioral Ecology 17:933–939.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Online at http://www.r-project.org/

Schoener, T.W. 1971. Theory of feeding strategies. Annual Review of Ecology and Systematics 2:369–404.

Shafir, S. & J. Roughgarden. 1998. Testing predictions of foraging theory for a sit-and-wait forager, *Anolis gingivinus*. Behavioral Ecology 9:74–84.

Unsicker, S.B., G. Kunert & J. Gershenzon. 2009. Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. Current Opinion in Plant Biology 12:1–7.

Willmer, P.G., C.V. Nuttman, N.E. Raine, G.N. Stone, J.G. Pattrick, K. Henson, P. Stillman, L. McIlroy, S.G. Potts & J.T. Knudsen. 2009. Floral volatiles controlling ant behaviour. Functional Ecology 23:888–900.